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## Variation in Coded Morphological Characters in the Japanese Common Toad from Momoyama, Kyoto, Japan

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**ABSTRACT** — Variation in 30 attribute characters was analyzed in the Japanese common toad, *Bufo japonicus japonicus*, from Momoyama, Kyoto, Japan. Extremely small, metamorphosed young quickly changed their morphology until they grew to be ca. 33 mm in SVL. Larger young showed no sexual dimorphism. Sexual maturity caused greater morphological divergence in males than in females, and adult males were distinct from the remaining sex/age groups in many characters, especially in wart characters. It is suggested that extremely small young, larger young, adult males, and adult females should be treated separately in analyses of coded morphological characters for taxonomic purposes.

### INTRODUCTION

Japanese toads, like other members of the *Bufo bufo* complex, are notoriously difficult to classify. Taxonomic revision from the analysis of morphometric characters has been made by Matsui [1]. As pointed out in that paper, some other morphological characters, such as body coloration and degree of toe webbing, have been regarded as important in the classification of Japanese toads [2-4], yet these characters are variable in relation to age and/or sex. Hitherto, no detailed studies have been made on these characters in the Japanese common toad nor in other Japanese anurans except for *Rana nigromaculata* and *R. brevipoda* (= *porosa*) [5].

This article presents the variation of coded morphological characters within a single population of *Bufo j. japonicus* (population 63 in [1]). It provides basic information for analyzing geographic variation among toads of the *Bufo bufo* complex from Japan and the Eurasian continent. To this end, variation relating to season, sex, and age was analyzed. This report also elucidates the general pattern of morphological differentiation of characters in this species.

### MATERIALS AND METHODS

The 122 Japanese common toads (*Bufo j. japonicus*) used in this study were collected in Momoyama, Fushimi-ku, Kyoto during 1972 and 1977. Data for each age/sex group regarding season of collection, number of sampled individuals, and body size are summarized in Table 1. Immediately after capture, records on coloration were made for most specimens. Records were again made after the specimens were preserved in 70% ethanol for comparison to other specimens. Determination of sex and maturity was made after Matsui [1]. Observations were made with the naked eye or under a binocular stereoscopic microscope at low magnification. The sample size of smaller young (i.e., less than ca. 35 mm in snout-vent length (SVL)) was too small for statistical analysis. For the other age/sex groups, Chi-squared or Fisher's exact tests [6] were run to detect the presence or absence of seasonal variation in adults, sexual dimorphism in adults and young, and age variation in both sexes, in the frequency distribution of individual toads among character states for 30 coded morphological characters (significance level=95%). Characters, their states, and corresponding codes (in parenthesis) are noted below. Most of these characters should ideally be analyzed as continuous variables [6] if more

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TABLE 1. Japanese common toads examined

Age	Sex	N	Months of collection	SVL range (mm)
Smaller young	?	9	late June — mid August	13.7 — 33.0
Larger young	M	26	late August — early October	49.2 — 90.3
	F	26	late August — early October	40.2 — 92.8
Adult	M	16	early March — mid March	79.8 — 116.5
	M	15	late August — early October	90.8 — 104.5
	F	11	early March — mid March	84.8 — 135.0
	F	3	June — July	105.0 — 115.2
	F	16	late August — early October	94.0 — 134.8

M=males; F=females.

pertinent method of measurement is available. However, this is hardly practiced at present, and therefore, I treated all the characters as attributes in this work. In defining these character states, those found among toad populations from localities other than Momoyama are included for future comparisons. *Character 1*, canthus: rounded (0); blunt (1); sharply edged (2). *Character 2*, parietal region: convex (0); flat (1); slightly concave (2); concave (3). *Character 3*, parotoid gland: not developed (0); flat (1); moderately elevated (2); strongly elevated (3). *Character 4*, tympanum: hidden (0); partially hidden (1); evident, not protruded (2); extremely evident, protruded (3). *Character 5*, tubercles on canthus: absent, skin smooth (0); small granules scattered (1); dense granules (2); large warts present (3); canthal ridge present (4). *Character 6*, tubercles on parietal region: states and codes same as character 5. *Character 7*, tubercles on dorsum: absent or with a few granules (0); tubercles moderately developed (1); tubercles strongly developed (2). *Character 8*, tubercles on forearm: states and codes same as character 7. *Character 9*, tubercles on tibia: states and codes same as character 7. *Character 10*, tubercles on venter: states and codes same as character 7. *Character 11*, tubercle row on flank: absent, skin smooth (0); weak row recognized (1); evident row, each tubercle separated (2); evident row, tubercles continuous (3). *Character 12*, conical tip on wart: tip absent (0); tips on limbs (1); tips on limbs and flank (2); tips on limbs, flank and venter (3); tips on limbs, flank, venter and dorsum (4). *Character 13*, horny layer on

wart: layer absent (0); layers on limbs (1); layers on limbs and flank (2); layers on limbs, flank and venter (3); layers on limbs, flank, venter and dorsum (4). *Character 14*, tarsal fold: fold absent (0); row of warts present (1); weakly defined fold (2); strongly defined fold (3). *Character 15*, toe webbing: poorly developed on all four points (inner edge of 5th toe, outer and inner edges of 4th toe, and outer edge of 3rd toe) (0); well developed on one of four points (1); well developed on two of four points (2); well developed on three of four points (3); well developed on four points (4). *Character 16*, dorsal coloration: dark brown (0); light brown (1); grayish brown (2); grayish yellow (3); yellowish brown (4). *Character 17*, dorsal marking: marking absent (0); light marking (1); dark marking (2). *Character 18*, spot behind parotoid: spot absent (0); weak spot (1); strong spot (2). *Character 19*, forelimb stripe: stripe absent (0); weak stripe (1); strong stripe (2). *Character 20*, tibial stripe: states and codes same as character 19. *Character 21*, loreal spot: states and codes same as character 18. *Character 22*, dark marking on sides of parotoid: marking absent (0); weak marking (1); strong brown marking (2); strong black marking (3). *Character 23*, dark stripe on flank: stripe absent (0); weak stripe (1); strong, partial stripe (2); strong continuous stripe (3). *Character 24*, light stripe on flank: stripe absent (0); weak stripe (1); strong, unbordered stripe (2); strong, clearly bordered stripe (3). *Character 25*, light marking on parotoid: marking absent (0); weak marking (1); strong, unbordered marking (2); strong, clearly

bordered marking (3). *Character 26*, mid-dorsal line: line absent (0); weak, partial line (1); clear, partial line (2); weak, continuous line (3); clear, continuous line (4); clear, wide stripe (5). *Character 27*, ventral dark marking: undefined, wide-spreading dark pigmentation (0); dark marking absent (1); a few markings (2); moderate number of markings (3); heavily mottled (4). *Character 28*, dark marking on throat: marking absent (0); weak marking (1); strong marking (2). *Character 29*, dark marking along lower jaw: marking absent (0); weak, partial marking (1); strong, interrupted marking (2); strong, continuous marking (3). *Character 30*, red marking: marking absent (0); weak marking or a few spots (1); strongly spotted (2); forming band on flank (3); body entirely reddish (4).

## RESULTS

The results of analyses on the frequency distribution of individuals for each character code are shown in Table 2 for each group divided by season of collection, age, and sex. The results of statistical comparisons within and among these groups are summarized in Table 3.

*Seasonal variation in adults* Presence of seasonal variation was compared between the samples collected in spring (=breeding season) and autumn for each sex of adults. Significant differences in two characters in males (development of tympanum and spot behind parotoid) and three in females (tubercles on canthus, tubercles on forearm, and conical tip on wart) were found: 1) character 4 in males: protruded tympanum, not found in autumn sample, was found in about 1/3 of spring sample; 2) character 18 in males: none of autumn sample had spot behind parotoid, but about half of the spring sample possessed a weak spot; 3) character 5 in females: individuals lacking tubercles and those with scattered small granules on canthus were similar in frequency in autumn sample, but the latter state was infrequent in spring sample; 4) character 8 in females: strongly developed tubercles on forearm were found in a larger number in the autumn sample, whereas the frequencies of moderately and strongly developed states were about the same in the spring sample;

5) character 12 in females: most individuals in the autumn sample possessed conical tips on the limb warts, but in the spring sample, individuals without conical tips were more numerous than those with tips on limb warts. Thus, females are considered to be seasonally more variable in wart characters, and warts and conical tips are less developed in spring.

*Sexual variation* As seen in Table 3, no sexual differences were found among the 30 characters in the young. On the contrary, many characters showed sexual dimorphism in adults, and 12 of 30 characters were found to be significantly different between the sexes (Table 3).

Among the 5 characters showing seasonal variation in adults, character 4 did not differ between the sexes in either the spring or autumn sample. Similarly, character 5 did not differ by sex between spring and autumn samples. By contrast, character 8 did differ by sex in both spring and autumn samples, the tubercles being more developed in the females than the males. Character 12 was sexually dimorphic in autumn samples and was more developed in females, but no difference was found in spring samples. Likewise, character 18 exhibited a sexual difference only in autumn samples, with males more frequently lacking the spot.

For the 25 characters showing no seasonal variation, spring and autumn samples were combined for analysis, with the result that the two sexes differed in the frequency of individuals for each of 9 characters: 1) character 3: males with flat parotoid were quite numerous, accounting for about 1/3 of the total, but there were few among the females; 2) character 6: most males lacked tubercles and had a smooth parietal region, whereas in females the number of individuals having a smooth parietal region and those with scattered small granules were about equal; 3) character 7: individuals with moderately developed tubercles predominated in males, whereas those with strongly developed tubercles on dorsum predominated in females; 4) character 13: a larger number of males lacked a horny layer, but more than half the females possessed a layer on limbs and flank; 5) character 15: in males, individuals with poorly developed webbing and those with webbing developed on one

TABLE 2. Distribution of individual toads among character codes of each character analyzed

Characters and codes																	
Character	1			2			3		4		5		6		7		
Code	0	1	2	1	2	3	1	2	2	3	0	1	0	1	0	1	2
M Y	0	3	23	14	10	2	1	25	25	1	6	20	3	23	1	6	19
F Y	0	1	25	19	7	0	1	25	26	0	3	23	0	36	0	5	21
M A Spr.	0	8	8	4	6	6	6	10	11	5	14	2	15	1	6	9	1
M A Aut.	0	9	6	0	11	4	4	11	15	0	12	3	11	4	3	9	3
F A Spr.	1	6	4	1	6	4	0	11	10	1	10	1	8	3	0	6	5
F A Sum.	0	2	1	0	3	0	0	3	3	0	3	0	2	1	0	2	1
F A Aut.	1	7	8	1	11	4	2	14	16	0	8	8	6	10	1	4	11

Characters and codes																			
Character	8			9			10		11			12				13			
Code	0	1	2	0	1	2	1	2	1	2	3	0	1	2	3	0	1	2	3
M Y	0	6	20	0	3	23	15	11	4	15	7	1	17	8	0	5	4	14	3
F Y	0	2	24	0	0	26	16	10	2	16	8	1	19	5	1	5	2	16	3
M A Spr.	9	5	2	2	8	6	13	3	5	4	7	13	3	0	0	11	4	0	1
M A Aut.	5	6	4	1	4	10	14	1	0	7	8	11	4	0	0	11	1	2	1
F A Spr.	0	5	6	0	2	9	11	0	3	3	5	7	4	0	0	5	2	4	0
F A Sum.	0	2	1	0	1	2	3	0	1	1	1	0	3	0	0	0	0	3	0
F A Aut.	0	1	15	0	0	16	11	5	1	7	8	2	14	0	0	6	0	9	1

Characters and codes																			
Character	14		15				16				17			18			19		
Code	0	1	0	1	2	3	0	1	2	4	0	1	2	0	1	2	0	1	2
M Y	24	2	22	4	0	0	2	19	4	1	9	14	3	2	7	17	1	19	6
F Y	23	3	16	9	1	0	2	22	1	1	5	15	6	3	10	13	1	19	6
M A Spr.	16	0	9	6	1	0	0	1	0	15	15	1	0	9	7	0	12	4	0
M A Aut.	14	1	6	8	0	1	0	0	0	15	15	0	0	15	0	0	11	4	0
F A Spr.	11	0	3	4	4	0	3	7	0	1	11	0	0	4	6	1	7	4	0
F A Sum.	3	0	3	0	0	0	3	0	0	0	3	0	0	1	1	1	2	1	0
F A Aut.	16	0	2	6	7	1	4	11	0	1	16	0	0	9	6	1	10	6	0

Characters and codes																		
Character	20			21			22	23			24				25			
Code	0	1	2	0	1	2	3	1	2	3	0	1	2	3	0	1	2	3
M Y	1	21	4	3	20	3	26	2	5	19	0	8	1	17	5	11	6	4
F Y	4	20	2	4	21	1	26	2	0	24	0	7	0	19	1	16	5	4
M A Spr.	11	5	0	12	4	0	16	0	4	12	0	3	3	10	2	12	1	1
M A Aut.	11	4	0	8	7	0	15	0	1	14	0	7	0	8	0	14	0	1
F A Spr.	7	4	0	8	3	0	11	0	1	10	0	2	2	7	0	6	3	2
F A Sum.	3	0	0	2	1	0	3	0	0	3	0	1	0	2	0	2	1	0
F A Aut.	12	4	0	8	7	1	16	0	0	16	1	4	2	9	2	10	2	2

(TABLE 2. Continued)

Characters and codes																				
Character	26					27				28			29				30			
Code	0	1	2	3	4	2	3	4	0	1	2	1	2	3	0	1	2	3		
M Y	15	6	4	0	1	10	13	3	21	4	1	0	4	22	14	3	4	5		
F Y	18	7	1	0	0	6	14	6	21	5	0	0	9	17	19	4	3	0		
M A Spr.	6	7	1	2	0	7	8	1	15	1	0	3	1	12	10	5	0	1		
M A Aut.	6	4	3	2	0	6	7	2	13	2	0	3	5	7	12	3	0	0		
F A Spr.	3	4	3	1	0	1	7	3	8	3	0	0	2	9	5	3	3	0		
F A Sum.	1	0	1	1	0	0	1	2	1	2	0	0	0	3	1	2	0	0		
F A Aut.	10	5	0	0	1	2	10	4	11	5	0	0	5	11	11	4	1	0		

Y=larger young, A=adults. Character codes to which no individuals belonged were omitted for some characters.

TABLE 3. Results of statistical comparisons showing sexual (M=males, F=females) and age (Y=larger young, A=adults) differences in 30 characters.

Character	Difference			
	Sexual (M/F)		Age (Y/A)	
	Y	A	M	F
1	NS	NS	++	++
2	NS	NS	++	++
3	NS	+	++	NS
4	NS	NS	NS*	NS
5	NS	NS	++	++*
6	NS	++	++	++
7	NS	++	++	NS
8	NS	++ ++	++	NS*
9	NS	NS	++	NS
10	NS	NS	+	NS
11	NS	NS	NS	NS
12	NS	NS ++	++	++*
13	NS	++	++	NS
14	NS	NS	NS	NS
15	NS	++	++	++
16	NS	++	++	+
17	NS	NS	++	++
18	NS	NS +	++*	++
19	NS	NS	++	++
20	NS	NS	++	++
21	NS	NS	++	++
22	NS	NS	NS	NS
23	NS	NS	NS	NS
24	NS	NS	NS	NS
25	NS	++	+	NS
26	NS	NS	NS	NS
27	NS	+	NS	NS
28	NS	++	NS	NS
29	NS	NS	NS	NS
30	NS	NS	NS	NS

NS:  $P > 0.05$ , +:  $P < 0.05$ , ++:  $P < 0.01$ .

Asterisks signify presence of within-group seasonal variation. For inter-sex comparisons of adults in characters 8, 12 and 18, results for spring (left) and autumn (right) samples are shown. Abbreviations as in Tables 1-2.

point were about equal in number, but in females about equal number of individuals had webbing developed on one or on two points, indicating better development of webbing than in males; 6) character 16: almost all males had a yellowish brown dorsum, while a larger number of females had a light brownish dorsum, and individuals with a dark brownish dorsum were limited to females; 7) character 25: females differed from males in the high frequency of individuals with strong marking on parotoid; 8) character 27: males had generally less developed ventral dark marking than females and were characterized by a high frequency of individuals with few markings; 9) character 28: most males lacked throat marking, but 1/3 of the females possessed it.

*Age variation* Due to the inadequate sample size for smaller young, morphological change in the stage from immediately after metamorphosis to ca. 35 mm SVL is simply described in Table 4. As seen in the table, as many as 19 of 30 characters differed between individuals captured within one month of metamorphosis (mean SVL=14.2 mm) and more advanced individuals having mean SVL of 21.2 mm. Between the latter individuals and further advanced individuals (mean SVL=32.5 mm), 13 character states differed, but these latter individuals in turn differed from larger young at most in 7 characters (character states for larger young were represented by the corresponding codes including the largest number of individuals). That is, morphological differentiation of a young toad by the SVL of about 32.5 mm is remarkable, but it becomes less marked thereafter.

When larger young and adults were compared for each sex, they differed significantly in 19 of 30 characters in males, whereas they differed in only 12 characters in females (Table 3). In 12 characters, both sexes differed in the frequency distribution of character codes between young and adults: 1) character 1: a larger number of young possessed a sharply edged canthus, while most adults had a blunt canthus; 2) character 2: most young had a flat parietal, but in adults a slightly concave parietal was most frequent and a concave parietal was more frequent than a flat parietal; 3) character 5 and 4) character 6: most young had canthus and parietal with scattered small granules

TABLE 4. Observed codes for 30 characters in young

Character	Character codes			
	Smaller young		Larger young	
	$\bar{X}$ SVL = 14.2 mm (N=3)	$\bar{X}$ SVL = 21.2 mm (N=3)	$\bar{X}$ SVL = 32.5 mm (N=3)	SVL = 40.2-92.8 mm (N=52)
1	0	2	2	2
2	0	1	1	1
3	0	1	1	2
4	0	1	2	2
5	1	1	1	1
6	1	1	1	1
7	0	1	2	2
8	0	1	2	2
9	0	1	2	2
10	1	1	1	1
11	1	1	2	2
12	0	2	1	1
13	0	2	2	2
14	0	0	0	0
15	1	0	0	0
16	0	0	1	1
17	2	0	1	1
18	0	0	2	2
19	1	2	1	1
20	1	2	1	1
21	1	2	2	1
22	1	3	3	3
23	1	1	2	3
24	1	0	1	3
25	1	1	1	1
26	0	1	1	0
27	0	3	3	3
28	0	1	1	0
29	1	1	3	3
30	0	0	0	0

Character codes for larger young are represented by modes.

or dense granules, but a larger number of adults had a smooth canthus and parietal; 5) character 12: individuals with conical tips on warts of limbs and flank were most abundant in young, but most adult males and about 1/3 of adult females lacked a conical tip on warts; 6) character 15: in most young, toe webbing was poorly developed in all four points, but individuals with more developed



webbing were more frequently observed in adults; 7) character 16: individuals with light brownish dorsum were most abundant in young, but in adults, most males had dorsum coloured yellowish brown and about 1/3 of females were dark brown; 8) character 17: a larger number of young had light dorsal marking, but most adults lacked marking; 9) character 18: more than half the young had a strong spot behind parotoid, but in adults, most males lacked the spot and females lacking the spot and those with a weak spot were nearly equal in number; 10) character 19 and 11) character 20: most young possessed weak forelimb and tibial stripes, but a larger number of adults lacked them; 12) character 21: a larger number of young had a weak loreal spot, but most adults lacked it.

In 7 characters, only males differed between young and adults: 1) character 3: most young had moderately elevated parotoid but flat parotoids were found in about 1/3 of adults; 2) character 7: in most young, tubercles on dorsum were strongly developed, but moderately developed tubercles were most common in adults; 3) character 8: most young had strongly developed tubercles on forearm, but nearly half the adults lacked tubercles; 4) character 9: tubercles on tibia were strongly developed in most young, whereas individuals with moderately developed tubercles frequently occurred among adults; 5) character 10: individuals with moderately developed tubercles on venter and those with strong tuberculation were nearly equal in number in young, but the former condition was most frequent in adults; 6) character 13: a larger number of young had horny layers on warts of limbs, flank, and ventrum, whereas adults usually lacked a horny layer; 7) character 25: individuals with weak marking on parotoid accounted for less than half the number in young while such individuals were most common among adults.

## DISCUSSION

From the above results, the pattern of morphological differentiation in the Japanese common toad (*Bufo j. japonicus*) can be summarized as follows: 1) just metamorphosed smaller young rather abruptly change their morphology in respect

to coded attribute characters by the time they reach ca. 33 mm SVL; 2) larger young are not sexually dimorphic in coded characters; 3) sexual maturity causes rather significant morphological change, and the degree is far greater in males than in females. The latter two points agree well with the results obtained for morphometric characters in this species [1].

Among remarkably variable characters were those related to development of warts and spines, and as shown in the results, adult males of *Bufo j. japonicus* usually have a smoother back than females and young. This seems to be a general trend in toads of the *Bufo bufo* complex since Liu [7], in analyzing secondary sex characters of Chinese anurans, reported that smooth skin was found in the male, whereas prominent spinous warts were found in the female of *Bufo b. japonicus* (=northern population of *Bufo gargarizans*), a close relative of the Japanese common toad. Smith [8] and De Lange [9] also noted that male European *Bufo bufo bufo* had smoother skins than females in the breeding season. A reverse situation, i.e., rough skin in the adult male, is universally found among toad species [10] and Taylor [11] stated that a change similar to that found in *Bufo japonicus* was unusual in toads. The latter trend, however, is not uncommon among remotely related *Bufo* species; In *B. funereus* from Upemba, Congo, the dorsal skin of females and young males is spinose, whereas that of breeding males is very smooth [12]. Changes in dorsal spinosity were demonstrated to be correlated with the development of the nuptial pads, i.e., sexual maturity [12].

The development of warts and conical spines was, however, seasonally variable even in females of the Japanese common toad, and samples collected in spring (=breeding season) included many individuals with poorly developed warts and spines like males. This fact seems to indicate the presence of some hormonal regulation similar to the one that would occur in males. The head of the young Japanese common toad has scattered granules, contrasting with the smooth head of adults. This result coincides well with the suggestion by Boring [13] that the smooth head in *Bufo bufo gargarizans* (= *Bufo gargarizans*) from China appears especially in old specimens.



In the Japanese common toad population from Momoyama, adult males had slightly more less-developed toe webbing than adult females. This is an unexpected situation since the closely allied European *Bufo b. bufo* usually has been reported to have better developed webbing in adult males [8, 14]. De Lange [9], on the contrary, stated that the relative dimensions of the webs and sex could not be correlated in that species. It has not been determined whether there is species-specific variation in the degree of toe webbing development.

The variability of the middorsal line was not related to sex or age in the Japanese common toad, and this result is compatible with that obtained by Martof and Humphries [15] for North American *Rana sylvatica*. They found that the middorsal white line in that species did not change ontogenetically, nor was it sex-linked. In their specimens the stripe was either fully present or entirely absent, and they attributed this phenomenon to the control of a single gene. By contrast, the appearance of the stripe was quite variable in the Japanese common toad, sometimes completely and sometimes partially present, and simple regulation of its expression by a single gene is doubtful in this species.

Adult females had the most developed ventral dark marking in the Japanese common toad. Opatrný [16] similarly found in *Bufo viridis* from Czechoslovakia that dark markings on the ventral surface appeared only in the later stages of ontogeny but that males are more often marked than females in this species. The presence of red marking was regarded as characteristic for toads from montane regions [3], but red spots were occasionally observed among individuals used in this study (from area less than 100 m in altitude) regardless of sex and age.

From the results reported here, it is concluded that 1) immature young except extremely small, just metamorphosed young can be treated without consideration of sex; 2) adults should be separated from young in some characters; 3) adult males and females should be separately treated in many characters, and 4) the collecting season should be considered in several characters in order to analyze geographic variation of coded attribute characters.

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